

WHAT IS CLAIMED IS:

1. A method comprising:
 - a) obtaining at least one sample suspected of containing one or more target cells or pathogens;
 - b) using the target cell or pathogen to generate pyrophosphate (PPi) by an enzyme-catalyzed reaction;
 - c) using the PPi to produce light by a luciferase dependent process;
 - d) accumulating the number of photons produced over a time interval; and
 - e) using the accumulated photons to detect the target cells or pathogens.
2. The method of claim 1, wherein the PPi is generated by a reaction catalyzed by an enzyme selected from the group consisting of a DNA polymerase, an RNA polymerase, a reverse transcriptase and a terminal transferase.
3. The method of claim 2, wherein the enzyme is thermostable.
4. The method of claim 1, further comprising producing ATP from the PPi.
5. The method of claim 4, wherein the production of ATP from PPi is catalyzed by ATP sulfurylase, FMN adenyltransferase, adenylyl transferase or glucose-1-phosphate adenyltransferase.
6. The method of claim 1, wherein the luciferase dependent process comprises a bioluminescence regenerative cycle (BRC).
7. The method of claim 6, wherein the BRC utilizes thermostable luciferase and/or ATP sulfurylase.
8. The method of claim 1, further comprising detecting a nucleic acid, an oligonucleotide or an aptamer.

9. The method of claim 8, wherein the nucleic acid is specific for a group, species or strain of pathogen.
10. The method of claim 9, wherein the pathogen nucleic acid is amplified.
11. The method of claim 10, wherein the nucleic acid amplification technique is selected from the group consisting of polymerase chain reaction (PCR) amplification, strand displacement amplification, Qbeta replication, transcription-based amplification (TAS), nucleic acid sequence based amplification (NASBA), one-sided PCR, RACE (rapid amplification or cDNA ends), ligase chain reaction amplification (LCR), 3SR (self-sustained sequence replication-reaction) amplification and rolling circle replication.
12. The method of claim 8, wherein the nucleic acid, oligonucleotide or aptamer binds to a protein.
13. The method of claim 12, wherein the protein is part of a target cell or pathogen.
14. The method of claim 12, wherein the protein binds to a target cell or pathogen.
15. The method of claim 14, wherein the protein is an antibody, antibody fragment, FAb fragment, genetically engineered antibody, monoclonal antibody, polyclonal antibody or single chain antibody, fusion protein, binding protein, receptor protein, enzyme, inhibitory protein or regulatory protein.
16. The method of claim 4, wherein the concentrations of ATP and PPi reach steady state levels.
17. The method of claim 16, further comprising integrating the light output over time during the steady state.
18. The method of claim 17, further comprising adding between 0.01 and 10 attomoles of ATP or PPi to the sample before light is produced.

19. The method of claim 2 or claim 6, wherein the luciferase, ATP sulfurylase, DNA polymerase, RNA polymerase, reverse transcriptase and/or terminal transferase are stable to at least 90°C for at least 10 minutes.
20. The method of claim 1, wherein sensitivity of detection is at least 0.1 attomol.
21. The method of claim 20, wherein 1000 target cells or pathogens can be detected in a sample.
22. The method of claim 1, further comprising determining the number of target cells or pathogens in the sample.
23. The method of claim 1, further comprising identifying the target cells or pathogens in the sample.
24. The method of claim 9, further comprising detecting a single nucleotide polymorphism (SNP) in the pathogen nucleic acid.
25. A method comprising:
 - a) obtaining at least one sample suspected of containing one or more target cells or pathogens;
 - b) binding the target cells or pathogens to a solid surface;
 - c) removing unbound cells or pathogens;
 - d) lysing the bound cells or pathogens; and
 - e) detecting endogenous ATP and/or PPi from the lysed cells or pathogens by a luciferase dependent process.
26. The method of claim 25, wherein the ATP and/or PPi is detected by BRC assay.
27. The method of claim 25, further comprising producing ATP from PPi.

28. The method of claim 27, wherein the production of ATP from PPi is catalyzed by ATP sulfurylase, FMN adenyltransferase, adenylyl transferase or glucose-1-phosphate adenyltransferase.
29. The method of claim 28, wherein the luciferase, ATP sulfurylase, FMN adenyltransferase, adenylyl transferase or glucose-1-phosphate adenyltransferase is thermostable.
30. The method of claim 26, further comprising
 - a) using a BRC assay mixture with at least one BRC enzyme or substrate inactivated by peptide linkage;
 - b) exposing the BRC assay mixture to the cell or pathogen lysate;
 - c) activating the inactivated BRC enzyme or substrate; and
 - d) producing light from the endogenous ATP and/or PPi.
31. The method of claim 30, wherein the lysate contains a protease and said protease removes the linked peptide from the BRC enzyme or substrate.
32. The method of claim 31, wherein the inactivated BRC enzyme is luciferase or ATP sulfurylase.
33. The method of claim 31, wherein the inactivated BRC substrate is luciferin or APS.
34. A method for cell or pathogen detection comprising:
 - a) generating pyrophosphate in a cell or pathogen dependent process;
 - b) using thermostable ATP sulfurylase and luciferase to produce light from the pyrophosphate; and
 - c) measuring the light output to detect the cell or pathogen.

35. The method of claim 34, wherein the pyrophosphate is generated by an enzymatic reaction.
36. The method of claim 35, wherein the enzyme is selected from the group consisting of a DNA polymerase, an RNA polymerase, a reverse transcriptase and a terminal transferase.
37. The method of claim 36, wherein the enzyme acts upon a nucleic acid, oligonucleotide or aptamer substrate.
38. The method of claim 37, wherein the nucleic acid is specific for a group, species or strain of pathogen.
39. The method of claim 37, wherein the nucleic acid, oligonucleotide or aptamer binds to a protein.
40. The method of claim 39, wherein the protein is part of a target cell or pathogen.
41. The method of claim 40, wherein the protein binds to a target cell or pathogen.
42. A method comprising:
 - a) obtaining at least one sample suspected of containing one or more pathogen nucleic acids;
 - b) adding labeled nucleotides to the one or more pathogen nucleic acids with a thermostable terminal transferase; and
 - c) detecting the labeled nucleic acids.
43. The method of claim 42, wherein the nucleotides are labeled with one or more fluorophores.
44. A method of cell or pathogen detection comprising:
 - a) attaching a target cell or pathogen to a substrate;
 - b) binding a first binding moiety to the target cell or pathogen;

- c) binding a second binding moiety to the first binding moiety, wherein the second binding moiety is attached to a dextran or dendromer molecule labeled with oligonucleotides;
- d) generating pyrophosphate by terminal transferase mediated addition of nucleotides to the oligonucleotides; and
- e) detecting the pyrophosphate.

45. The method of claim 44, wherein the pyrophosphate is detected by BRC assay.

46. A system comprising:

- a) one or more reaction chambers;
- b) a microfluidic system;
- c) one or more photodetectors
- d) a thermostable luciferase; and
- e) a thermostable ATP sulfurylase.

47. The system of claim 46, wherein each chamber comprises one or more binding moieties specific for a target cell or pathogen.

48. The system of claim 47, wherein the binding moieties are attached to a hydrogel.